PB86-163342

Methodology for the Determination of Dinitroaniline Herbicides in Tissue and Excreta

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before complet

1. REPORT NO.	2.	3. PB86-163342
EPA/600/J-85/318		

4. TITLE AND SUBTITLE
Methodology for the Determination of Dinitroaniline Herbicides in Tissue and Excreta
(Journal Version)

5. REPORT DATE
1985

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Thomas R. Edgerton, William Scott, Ralph E. Linder

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Health Effects Research Laboratory
Research Triangle Park, NC 27711

10. PROGRAM ELEMENT NO.
ACAE1A

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA - 600/11

15. SUPPLEMENTARY NOTES
Journal of Analytical Toxicology, 9:15-19, January/February 1985

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	6
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

Journal of Analytical Toxicology, Vol. 9, January/February 1985

EPA/600/J-85/318
JOURNAL ARTICLE

Methodology for the Determination of Dinitroaniline Herbicides in Tissue and Excreta*

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Abstract

A method is presented for the analysis of trace amounts of dinitroaniline herbicides in tissue and excreta. The method employs extraction of the tissue or excreta with organic solvent, clean up by liquid/liquid partitioning or silica gel chromatography, and ultimate analysis by gas chromatography using electron capture detection. Recoveries of greater than 80% were noted over the range of concentration 0.1 to 1.0 μg/g.

Introduction

The dinitroaniline (DNA) herbicides (trifluralin, benefin, profluralin, fluchoralin, butralin, isopropalin, pendimethalin) are a group of selective pre- and post-emergence herbicides used on a wide variety of crops for the control of annual grasses and broadleaf weeds. The herbicides are often applied aerially.

There are FDA methods for the analysis of DNA herbicides in a variety of crops. Methods for crop residues are also reported in the literature (1-3). These methods basically involve extraction of the DNA residues from the sample substrate with appropriate solvents (benzene, methanol, acid/methanol) and cleanup by absorption chromatography using either Florisil® or alumina. Electron capture/gas chromatography (EC/GC) is used as the determinative step. The metabolism of DNA herbicides in animals has been reported (4-5). In addition, photodecomposition studies (6-11) and plant, soil, and microbial metabolism studies (12-19) are also found in the literature. The literature contains no reference to methods for determining DNA residues in animals or man.

Because of the possibility of agricultural worker exposure or drift from aerial application in residential areas, a selective and quantitative method for the determination of DNA residues has been developed for adipose tissue, liver, blood, urine, and feces. This method can be applied to humans when suspected exposure occurs.

The first step in the analytical methods for urine, blood, and feces consisted of extraction with organic solvent. The sample extract was then cleaned up by silica gel chromatography and analyzed by EC/GC. Liver and adipose tissue were extracted with organic solvent, cleaned up by liquid/liquid partitioning, and then passed through silica gel prior to EC/GC analysis.

Materials and Methods

Animal Treatment

Male 60-day-old Sprague-Dawley rats (273-326 g) were distributed into three groups of three rats each. Solutions containing 4% analytical standard fluchloralin or pendimethalin were prepared by dissolving the compounds in corn oil. The solutions were administered by oral gavage in a relative dose volume of 5 mL/kg body weight to give daily dosages of 200 mg/kg fluchloralin or pendimethalin. The third group received corn oil only.

The rats were housed in individual metabolism cages with food and water provided *ad libitum*. Urine and feces were collected daily. Blood was collected from the abdominal aorta after anesthetizing the animals with ether. The animals were killed by exsanguination, and liver and perirenal fat samples were taken.

Apparatus

A model 222 gas chromatograph (Tracor) equipped with a nickel-63 electron capture detector, was operated in the pulsed linearized mode. Borosilicate glass columns (1.8 m × 4 mm i.d.) were packed with 80-100 mesh Chromosorb W coated with 3% OV-1, 5% OV-210, 1.5% OV-17/1.95% OV-210, or supported-bonded Carbowax 20M (SB-CW20M). The SB-CW20M column was operated at 170°C; the OV-1 and OV-17/OV-210 columns were operated at 190°C; and the OV-210 at 200°C, all with a 5% methane in argon carrier gas flow-rate of 60 mL/min.

*This paper has been reviewed by the Health Effects Research Laboratory U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Analytical results were confirmed on a model 3920B gas chromatograph (Perkin-Elmer) equipped with a nitrogen-phosphorus thermionic detector and equipped with a SB-CW20M.

Reagents and Materials

Anhydrous, granular sodium sulfate was soxhlet extracted for 4 hrs with hexane and oven dried at 130°C.

Silicar CC4 (Mallinckrodt, Inc.) was washed in a buchner funnel with methanol, acetone, toluene, and hexane and oven dried at 130°C.

All solvents were pesticide quality or equivalent.

Trifluralin (99.5%), butralin (99 + %), isopropalin (99.4%), fluchloralin (99.5%), benfin (100%), and pendimethalin (99.5%) were obtained from the EPA Pesticide Repository, Research Triangle Park, North Carolina.

Preparation of Standard Solutions

A standard of each dinitroaniline herbicide was prepared in acetone and stored at −15°C in a brown glass bottle. Sample fortifications were made from acetone dilutions of the seven DNA standards as a mixture.

Preparation and Elution of Silicar CC-4 Column

A size 22-9 Chromaflex column (Kontes) was loosely plugged with a small amount of glass wool. Silicar CC-4 (4 g) was added in small increments with tapping. Anhydrous, granular Na_2SO_4 (1.6 g) was removed from a drying oven and added to the top of the Silicar CC-4. The column was allowed to cool to room temperature. The column was pre-wetted with 10 mL of hexane and the eluate discarded. The sample was transferred onto the Silicar CC-4 column with the aid of a disposable pipet. The sample tube was rinsed with two 0.5-mL portions of hexane and transferred to the top of the Silicar CC-4 column. An additional 8.5 mL of hexane was added and the eluate discarded. The DNA residues were eluted from the column with 15 mL of 60% toluene in hexane.

Extraction Procedure for DNA Residues from Urine, Blood, and Feces

Urine (4.0 mL), blood (2 g), or feces (2 g) was placed in a 15 × 125-mm screw-cap culture tube. To this was added 3 mL of hexane. The tube was sealed with a Teflon-lined screw cap and shaken for 2 min. The sample was centrifuged and the organic layer transferred to a 15-mL centrifuge tube. This extraction was repeated twice more and the organic extracts combined with the first 3 mL extract. The entire extract was concentrated to 0.5 mL using an N-EVAP analytical evaporator.

Extraction Procedure for DNA Residues from Liver and Adipose

Liver (500 mg) or adipose (500 mg) was extracted with three 3-mL portions of acetone in a size 22 or 23 tissue grinder (Duall). The sample was centrifuged after each extraction and the supernatants were combined in a 40-mL screw cap centrifuge tube. Twenty-five milliliters of 2% aqueous sodium chloride was added to the combined supernatants and mixed. The aqueous acetone mixture was extracted with two 5-mL portions of hexane. The extracts were combined in a 15-mL centrifuge tube and concentrated to a volume of 0.3-0.5 mL under a gentle stream of nitrogen.

Results and Discussion

Figure 1 illustrates the structures and lists accepted names for the seven DNA herbicides used in this study. Table I lists relative retention data for the seven DNA herbicides under study on four different GC columns. A support-bonded Carbowax 20M column was chosen for analysis of the DNA herbicides because of its separation capabilities, low column oven temperature, and speed of analysis.

Recoveries of the seven DNA herbicides through the silica gel column are listed in Table II. An upper limit of 1 g of each DNA herbicide standard was established as maximum level for column cleanup. Levels above 1 g were easily chromatographed after dilutions of the sample extract. In the DNA dosing study, no silica gel cleanup was required on adipose and feces sample extracts.

As shown in Table III, recoveries of DNA herbicides from fortified rat tissues and excreta averaged better than 80%. Method sensitivity is estimated at 0.1 ppm for tissue samples and excreta.

Silica gel (Silicar CC-4) was chosen for column chromatography because of its ability to remove co-extractives and still allow for quantitative recovery of the DNA residues. Basic alumina did give group separation of the DNA herbicides;

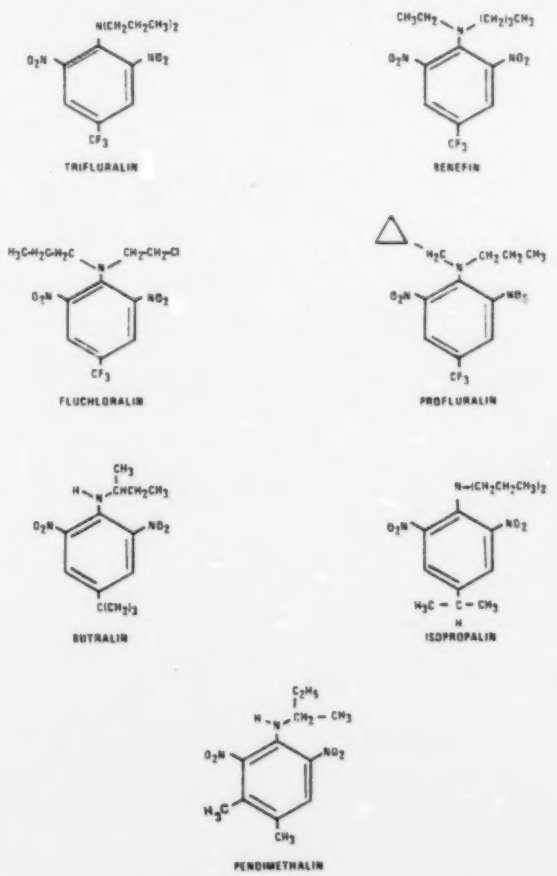

Figure 1. Structures of dinitroaniline herbicides.

Table I. Relative Retention Data for Dinitroaniline Herbicides

Compound	SB-CW20M (170°C; 60 mL/min) Minutes	SB-CW20M (170°C; 60 mL/min) RRT Aldrin	15% OV-17/1.95% OV-210 (190°C; 60 mL/min) Minutes	15% OV-17/1.95% OV-210 (190°C; 60 mL/min) RRT Aldrin	3% OV-1 (190°C; 60 mL/min) Minutes	3% OV-1 (190°C; 60 mL/min) RRT Aldrin	5% OV-210 (200°C; 60 mL/min) Minutes	5% OV-210 (200°C; 60 mL/min) RRT Aldrin
Trifluralin	1.97	0.58	3.35	0.41	2.01	0.37	2.91	0.95
Benefin	2.05	0.60	3.35	0.41	2.04	0.37	3.07	1.00
Profluralin	2.91	0.86	4.84	0.59	3.74	0.52	4.06	1.32
Fluchloralin	4.80	1.43	6.77	0.83	3.94	0.55	5.98	1.95
Butralin	5.98	1.77	13.74	1.69	8.19	1.14	8.90	2.90
Isopropalin	7.95	2.35	13.98	1.71	8.58	1.19	7.80	2.54
Pendimethalin	8.43	2.49	16.02	1.97	8.78	1.22	7.98	2.60

Table II. Recoveries of Dinitroaniline Herbicides from Silicar Column

Compound	Amount added (μg)	Amount recovered (μg)	Recovery
Benefin	1.000	0.921	92.1
	0.100	0.088	88.0
Profluralin	1.000	0.947	94.7
	0.100	0.091	91.0
Butralin	1.000	0.960	96.0
	0.100	0.092	92.0
Pendamethalin	1.000	0.951	95.1
	0.100	0.089	89.0
Trifluralin	1.000	0.940	94.0
	0.100	0.091	91.0
Fluchloralin	1.000	1.000	100.0
	0.100	0.091	91.0
Isopropalin	1.000	1.001	100.1
	0.100	0.097	97.0

however, the recoveries were low and erratic. The DNA herbicides could be recovered quantitatively from Florisil with acetone. The use of acetone as an eluting solvent resulted in the elution of GC interferences along with the DNA residues, thus making Florisil unacceptable.

The choice of extraction solvent for liver and adipose was found to be critical. Hexane extraction yielded recoveries of less than 30%. Acetonitrile, diethyl ether, toluene, plus various mixtures of these solvents gave similar low recoveries. Acetone was found to give the most reproducible quantitative recoveries.

Figures 2 and 3 are representative chromatograms of DNA standard mixture and liver extract from recovery studies following liquid/liquid partitioning and silica gel column cleanup. In Figure 2, the co-elution of trifluralin and benefin and the poor separation of isopropalin from pendimethalin should be noted. Because of these chromatographic problems, samples were fortified in two groups for recovery studies: group 1, benefin, pro-

Table III. Recoveries of DNA Herbicides from Fortified Tissues and Excreta*

Sample	PPM Added	Benefin	Profluralin	Butralin	Pendimethalin	Trifluoralin	Fluchloralin	Isopropalin
Adipose	0.1	74-83	78-91	78-90	78-91	66-80	79-90	69-82
	0.5	77-89	66-69	74-81	71-78	76-93	80-95	76-89
	1.0	85-94	68-70	83-88	82-86	78-90	79-92	73-93
	X	84.3	73.0	82.4	80.7	81.3	84.6	79.8
	S.D.	±5.7	±8.4	±4.6	±5.0	±7.6	±5.5	±7.5
Liver	0.1	81-97	83-95	78-86	81-88	78-91	77-87	81-97
	0.5	79-86	71-87	79-92	81-91	78-88	88-91	78-94
	1.0	83-90	72-97	90-101	81-93	81-93	82-91	78-94
	X	85.4	84.0	87.1	86.3	85.7	86.1	86.7
	S.D.	±4.8	±7.8	±7.2	±4.3	±4.5	±5.2	±6.0
Blood	0.1	82-91	81-90	83-91	89-94	83-95	84-101	85-93
	0.5	78-92	83-86	93-97	90-94	80-91	91-98	75-81
	1.0	78-92	82-94	78-96	74-95	75-95	86-100	81-97
	X	84.4	85.5	90.1	90.2	86.5	92.5	85.5
	S.D.	±5.1	±3.5	±6.3	±6.2	±6.9	±5.4	±6.6
Urine	0.1	81-92	82-92	93-100	92-95	81-92	91-100	89-98
	0.5	72-91	75-96	87-103	80-105	84-93	99-105	84-92
	1.0	94-99	94-97	97-102	99-100	90-99	93-102	96-101
	X	87.8	88.4	95.9	94.3	89.9	98.9	92.9
	S.D.	±8.4	±7.8	±5.1	±7.1	±5.0	±3.6	±4.9
Feces	0.1	77-95	85-96	73-95	75-96	84-93	80-89	78-93
	0.5	84-93	87-95	90-95	88-100	84-94	86-94	84-91
	1.0	89-94	92-97	86-110	80-106	88-94	83-110	88-96
	X	89.3	91.6	90.8	90.7	89.8	89.3	88.2
	S.D.	±4.9	±3.7	±8.5	±8.7	±3.8	±7.9	±5.5

*Four determinations each level

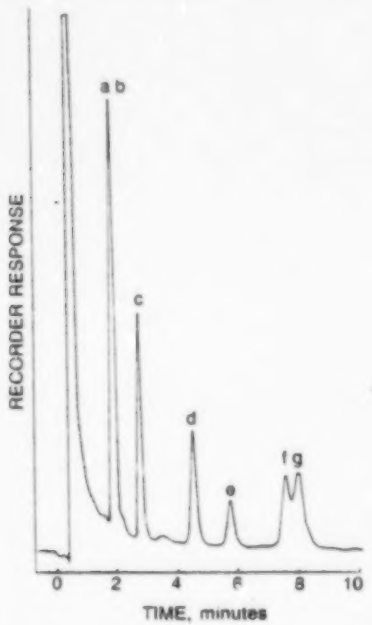

Figure 2. Gas chromatogram of standard dinitroanaline mixture. (a,b) Trifluralin and benefin; (c) profluralin; (d) fluchloralin; (e) butralin; (f) isopropalin; (g) pendimethalin. Column: SB-CW20M on 80/100 mesh Chromosorb W (AW). Oven temperature, 170°C. Five percent methane in argon; flow-rate 60 mL/min; Electron Capture Detector.

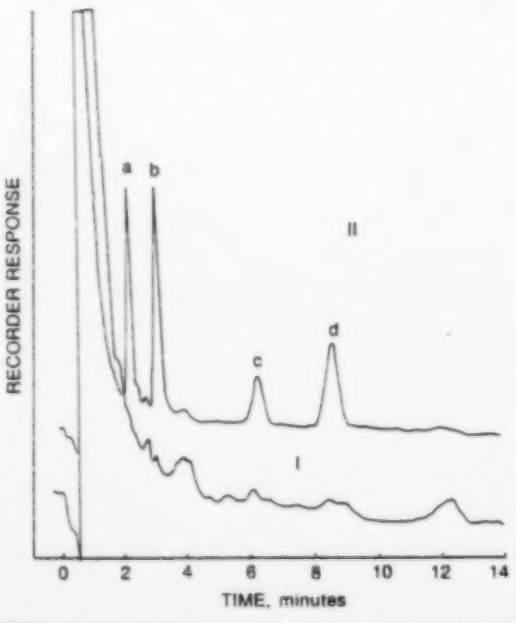

Figure 3. Gas Chromatograms of (I) control liver; injection, 63 mg tissue equivalent; (II) fortified liver extract (0.5 ppm); injection, 25 mg tissue equivalent (a) benefin; (b) profluralin; (c) butralin; (d) pendimethalin. Column: SB-CW20M on 80/100 mesh Chromosorb W (AW). Oven temperature, 170°C. Five percent methane in argon; flow-rate 60 mL/min. Electron Capture Detector.

fluralin, butalin, pendimethalin; group 2, trifluralin, fluchloralin, isopropalin.

Figure 4 is a representative chromatogram of a standard DNA mixture using a Nitrogen Phorphorus (NP) detector. With temperature programming, the DNA compounds are separated. Because of the lack of sensitivity of the particular NP instrument, it was only used for confirmation of the particular DNA residues.

Results of DNA residues in tissues and excreta from dosing studies are listed in Table IV. No attempts were made at this time to identify metabolites or to examine excretory storage dose relationships.

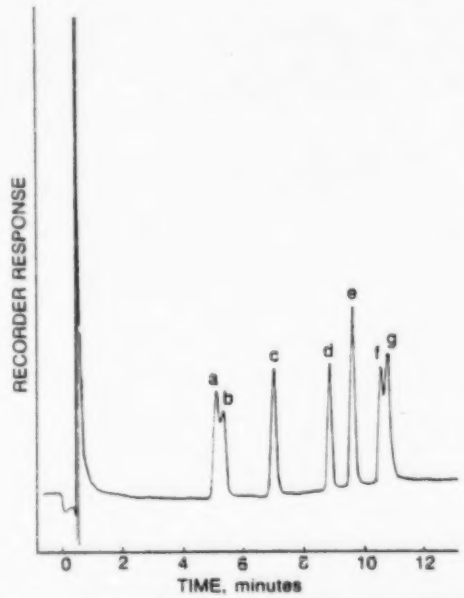

Figure 4. Gas chromatogram of standard dinitroaniline mixture. (a) Trifluralin; (b) benefin; (c) profluralin; (d) fluchloralin; (e) butralin; (f) isopropalin; (g) pendimethalin. Column: SB-CW20M on 80/100 mesh Chromosorb W (AW). Temperature programmed, oven initial temperature 145°C isothermal, 4 min, program 8°/min to final 190°C. Helium flow-rate 44 mL/min, Nitrogen Phosphorous Detector.

Table IV. Fluchloralin and Pendimethalin Residues

Sample No.	Adipose	Liver	Blood	Urine	Feces
		Fluchloralin Residues (ppm)			
9520	3.72	<0.10	<0.10	2.44	500.00
Control	<0.10	<0.10	<0.10	<0.10	<0.10
9521	137.20	0.45	<0.10	0.26	305.50
9522	154.70	<0.10	<0.10	<0.10	394.20
Control	<0.10	<0.10	<0.10	<0.10	<0.10
		Pendimethalin Residues (ppm)			
9523	22.89	<0.10	<0.10	2.50	1700.00
Control	<0.10	<0.10	<0.10	<0.10	<0.10
9524	26.2	<0.10	<0.10	0.93	212.00
9525	24.2	<0.10	<0.10	1.75	226.70
Control	<0.10	<0.10	<0.10	<0.10	<0.10

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Manuscript received April 12, 1982;
revision received May 29, 1984.